

BIOLOGICAL OBSERVATIONS ON THE
ORIENTAL RAT FLEA, *Xenopsylla cheopis*
(ROTHSCHILD), WITH SPECIAL STUDIES
ON THE EFFECTS OF THE
CHEMOSTERILANT TRIS (1-AZIRIDINYL)
PHOSPHINE OXIDE

By
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INTRODUCTION

The oriental rat flea, Xenopsylla cheopis (Rothschild), is recognized as a pest all over the world (Buxton, 1941, and Atlas of Plague, 1952). It can be found on ship rats throughout the year (Newstead and Evans, 1921). Hirst (1927) gave the following account of its dispersal:

X. cheopis is able to develop apart from its hosts' nest, so that it is capable of being much more easily dispersed in material such as grain, the debris of which affords nourishment for the larvae. Thus grain not only serves as a means of transport for rats and fleas from place to place, but is the most suitable medium for the multiplication of the most efficient insect vector of plague, X. cheopis, and the principal carrier of both disease and flea, the grain-eating Mus rattus.

According to Wu et al. (1936), plague, Pasteurella pestis (Lehman and Neumann), was first recorded in the sixth century A.D., starting in Egypt in 542, and finally spreading to Constantinople. This pandemic lasted for almost sixty years, and approximately 100,000,000 people died. In Europe in 1348, a plague pandemic, termed "the Black Death," killed 25,000,000 people (Herms, 1961). The plague pandemic which began in Hong Kong in 1894 was carried to many parts of the world through trade routes. Herms (1961) states that rats, infected by rat fleas, and transported in commercial goods, are the chief spreaders of the disease, with the plague likely to appear in a city "far removed from the original focus of infection."

From 1900 to 1952, cases of plague were recorded in thirty-nine countries (Atlas of Plague, 1952). In twenty-seven of these countries X. cheopis was one of the principal vectors. From 1945 to 1952, there were thirty-seven cases reported from the seaports of various countries.

Plague in the United States was first reported from San Francisco in 1900 (Herms, 1961, and Jellison, 1959). The epidemic ended in 1904, but reappeared in 1907. Other cities which have suffered from the disease include Seattle in 1907, New Orleans in 1912, several Gulf Coast cities in 1920, and Los Angeles in 1924.

The theory that rats in commerce are the principal carriers of plague would seem to be confirmed by the fact that most of the recorded plague epidemics began in seaports.

At present, sylvatic plague is endemic throughout the western United States, and in the provinces of Alberta and Saskatchewan, Canada. It has been determined from thirty-eight species of rodents and lagomorphs. According to Meyer (1947), the most prolific carriers of plague are the Sciuridae. It was discovered in 1908 that plague was no longer confined to rats and rat fleas in North America, but had become established in the ground squirrel, Citellus beecheyi (Richardson) and in its fleas. McCoy (1910) reported on plague in ground squirrels in California. He warns:

In the suburbs of towns and cities, rats and squirrels come into very close contact and it would seem very easy to have the disease carried from the squirrels to the rats, and as a result have a general infection of the cities.

X. cheopis is one of the vectors of murine typhus, Rickettsia mooseri Montiero, transmitted by fleas, with the rat and squirrel populations serving as reservoirs between epidemics. The cycle in this

instance is from animal to flea to animal to flea to --now and then-- man. The body louse, Pediculus humanus humanus Linnaeus, carries European typhus directly to man, at the cost of fatal infection to the louse itself. The whole series of Rickettsial diseases, superficially so much alike, differ dramatically when considered epidemiologically. The transmission mechanism varies considerably from one disease to the other. There is no common vector, although piercing-sucking arthropods are invariably involved. There is even less uniformity in the degree to which an animal reservoir is necessary to keep the infection alive, and in whether or not the vector itself succumbs to the disease. Chemo-sterilant programs would seem to be ideally suited for work against all types of the Rickettsiae.

The tapeworm, Hymenolepis diminuta (Rudolphi), the larvae of which develop in X. cheopis and other fleas, can complete its life cycle in man or in any susceptible animal which might ingest the infected flea.

Up to the present time, X. cheopis has been controlled only through the use of insecticides or control of the rodent host. With the advent of radiation and chemosterilants possible eradication of this and similar species can be envisaged.

No literature could be found relating to this type of work, but literature concerning radiation and chemosterilants used on other species of insects was studied so that a basic, practical pattern of research could be planned.

Although radiation was considered as a possible effective means of sterilization, one would not like to release thousands of fleas in any given area. Since fleas are pestiferous whether or not they are diseased, there is definitely an advantage in setting up stations for

sterilizing rats. Before the initiation of such a program, however, it would be essential to know what effects the chemosterilant might have on the fleas of the rat, especially whether or not feeding on the sterilized rat would in turn sterilize the flea. It was decided, therefore, to test the effects of the chemosterilant tepa on the Oriental rat flea.

X. cheopis, as is obvious from the preceding account, is still among the most important potential enemies of mankind. The basic purpose of this study, therefore, was to determine if the flea can be sterilized, if its sterility and mortality doses are close, and how it is affected by the chemosterilant tepa.

Since the effective use of chemosterilants depends upon a thorough study of the species to be sterilized, the literature was reviewed, and careful observations were made concerning the biology of X. cheopis.

REVIEW OF LITERATURE

Chemosterilants

Radiation-imitating chemicals, termed "radiomimetic" by Dustin (1947), have been known for some years. It was not until the Second World War, however, with work on poison gases, that they were shown to be possible cancer cures (Alexander, 1960). The radiomimetic compounds, as in radiation, cause the following cellular effects:

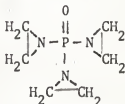
(1) mutation of genes and permanent changes in chromosomal structures; (2) interference with cell division processes which can cause death of the cell; (3) outright death of certain types of cells; (4) cancerous growths.

Fahmy and Fahmy (1958) found that alkylating agents (radiomimetic compounds) cause an amount of small deficiencies almost double those of mutagenically equivalent doses of X-radiation. These deficiencies are caused by failure in gene reproduction in situ, and not by chromosome breakage and reunion as in radiation. Alexander and Stacey (1958) suggest that the difference in biological action between radiation and radiomimetic compounds is that the latter have to diffuse into the cell making some of the DNA (deoxyribonucleic acid) molecules more accessible to the compound than are others.

A chemical which causes sexual sterility is called a chemosterilant. Weidhaas (1963) defines it further:

---the term chemosterilant is restricted to compounds which prevent production of sperm or ova, kill sperm or ova that have already been produced, or damage chromatic or genetic material in the sperm or ova so that zygotes, if formed, do not develop into mature progeny.

Two groups of chemical compounds which have shown great promise as sterilants of insects are the antimetabolites and the alkylating agents (LaBrecque, 1963 and 1965). An alkylating agent, the chemosterilant tepa causes replacement of a hydrogen atom with an alkyl group on an organic molecule. Its structural formula is the following:



According to Duvall (1960), tepa is very hygroscopic, being "extremely soluble" --although unstable-- in water, as well as being "very soluble" in alcohol, in ether, and in acetone. Solutions of tepa and one of the three diluents kept at about 5°C will remain comparatively stable for a week. Duvall reported the LD₅₀ in mice to be 47.0 mg/kg/d by mouth, the maximum tolerated single dose being 75.0 mg/kg/d.

Hayes (1964) reported that rats injected intraperitoneally with a dosage of 0.2 mg/kg/d of tretamine (2,4,6-tris(1-aziridinyl)-5-triazine) remained sterile up to at least 8 weeks after the last dose. An intraperitoneal dosage of 0.05 mg/kg/d of tretamine caused sterility in male rats. Fertility returned 3-4 weeks after the last dosage. Sexual behavior of the sterilized male was normal, as was the number and motility of the sperm.

The compounds presently available are not very stable (Smith, 1963a, and Hayes, 1964), and more information is needed as to their toxicity and application before expanded programs can be undertaken (Smith, 1963b; Smith et al., 1964; and Barnes, 1964).

Mitlin et al. (1957) obtained sterilization in house flies by using mitotic poisons. Three of the four chemicals tested usually inhibited oviposition, and prevented ovarian growth.

In 1958, LaBrecque began screening chemical compounds for their chemosterilizing effects. Of 2,000 compounds initially tested, 5 caused sterility in the house fly, Musca domestica Linnaeus, when placed in its food.

By 1963, 2,000 chemicals had been tested, 40 of them causing sterility, according to LaBrecque (1963). LaBrecque reported further in 1965 that 112 chemicals have been found to produce sterilant effects.

Several field tests have been conducted against the house fly with the chemosterilants tepa (tris(1-aziridinyl)phosphine oxide), metepa (tris(2-methyl-1-aziridinyl)phosphine oxide), and apholate (2,2,4,4,6,6-hexakis(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine). In a refuse dump at Bahia Honda Key, Florida, LaBrecque et al. (1962) conducted the first field test using tepa against the house fly. As a result, the adult fly populations were reduced from 47 to zero per grid count within 4 weeks with the use of cornmeal baits containing 0.5% tepa. Female flies trapped at the dump were checked for egg masses and viability. Egg masses had decreased from 100-10% within 4 weeks, and within 5 weeks egg viability had decreased to 1%. Metepa at 0.5% was applied in bait to droppings in a poultry house for control of the house fly (LaBrecque et al., 1963) with similar results.

In 1963, Gouck et al. conducted a test in a refuse dump at Pine Island, Florida, using a cornmeal bait of 0.75% apholate. They obtained sporadic results probably due to introduction of fresh flies from garbage trucks. Nevertheless, a reduction of flies did occur from 68 per grid count to between 5 and 20 for the first 7 weeks.

When bait was made available continuously, the population then decreased to between 3 and 0 per grid count.

Morgan and LaBrecque (1962 and 1964b) reported on the effects of apholate, tepa, and metepa on the ovarian development of house flies. In general, these compounds were found to inhibit ovarian development. The chromatin of the nurse cell nuclei was clumped in irregular masses.

LaBrecque et al. (1966) achieved 99-100% sterility in the male house fly at concentrations of hempa (hexamethylphosphoramide) as low as 0.25%. Female sterility was often as high as that of the male, although it varied.

Through the use of gamma radiation the screw-worm fly was eradicated from the island of Curacao, and from the southeastern United States. Recent research has been directed toward the use of chemosterilants to control this fly (Knippling, 1962). Chamberlain (1962) obtained sterility in adult screw-worm flies with apholate; however, he achieved only partial sterility in the pupae. In 1963, Crystal induced sterility in the same fly, with antimetabolites and with alkylating agents. Of 29 compounds tested, 26 caused sterility when incorporated into the diet, and 12 by topical application. In 1965, he further reported that the chemical N,N'-tetramethylenebis(1-aziridine-carboxamide) was far superior to gamma radiation in sterilizing the screw-worm fly.

The yellow-fever mosquito, Aedes aegypti (Linnaeus), and the common malaria mosquito, Anopheles quadrimaculatus Say, can be sterilized with chemosterilants in either the larval or the adult stage (Weidhaas et al., 1961; Weidhaas, 1962; Dame and Ford, 1964a; and Dame et al., 1964b,c). Apholate concentrations as low as 0.1%, when fed in the diet, reduced to zero the fertility of eggs laid by A. aegypti, while 0.5% completely eliminated egg fertility in A. quadrimaculatus. When larvae of A. aegypti were treated with 10 p.p.m. of tepa, all of the emerging adult males, and almost all of the females, were sterile. Apholate at 10 p.p.m. was not effective. Residual tests demonstrated that tepa at 680 $\mu\text{g}/\text{ft}^2$ gave complete sterility in both species of mosquito.

Glancey (1965) reported that, when fed in a honey solution, 0.5% hempa induced 100% sterility in female A. aegypti, and a 0.1% concentration produced 97% sterility in the male. He also found that residual deposits of 200-500 mg/ft^2 for 4 hours produced 90% sterility in the male.

Apholate affects the female A. aegypti reproductive system almost the same as in house flies (Rai, 1964 and 1965). Rai stated that the egg follicles of treated females were underdeveloped, and eventually degenerated; in some instances the follicular epithelium, eggs, and nurse cells degenerated.

Burden and Smittle (1963), testing twelve different chemosterilants on the German cockroach, Blattella germanica (Linnaeus), found that some of the compounds caused the oöthecae to be deformed, resulting in delayed and/or reduced hatch. Smittle (1964) conducted tests with tepa on the reproductive organs and embryogeny of this cockroach. He found

that 4 and 5 μg of tepa injected into adult male roaches produced complete sterility, although it inhibited neither mating nor sperm motility. Egg hatch was reduced 96% when 5 μg of tepa was injected into seventh-instar female nymphs. This chemosterilant caused the male testes to atrophy, and the basal oöcytes of females to be smaller than those of controls. Females mated with sterile males produced oöthecae; however, no hatch occurred.

Testes and ovaries of the eye gnat, Hippelates pusio Loew, were reduced in size when treated with tepa, metepa, or apholate (Schwartz, 1965). The germarium of the female ovarioles was the most severely affected; the germarial region of the male, void of spermatogonia, was almost collapsed. Sterility of 99-100% was obtained with 0.01% tepa, 0.1% metepa, and 0.5% apholate.

Tepa at 10 mg/ft² affected bird malaria, Plasmodium gallinaceum Brumpt, in A. aegypti when the mosquito was subjected to the residue either before or after feeding on infected chicks (Altman, 1963). The chemical caused reductions in the number of infected mosquitoes, the mean oöcyst count, and the rate of transmission.

Biology

The adult

Hirst (1926) was the first to report that emergence of X. cheopis from its cocoon is stimulated by vibration. The vibration can be mechanical, but breathing on the cocoons will also cause the adults to emerge.

Temperature and humidity.--At lower temperatures, unfed adults live longer than at higher ones. Buxton (1948) concluded that at temperatures from 24-32°C, the adult flea could survive.

The life cycle of the flea will vary according to the temperature and humidity. Hopkins (1935) reported that the life cycle could be accomplished in about 56-63 days, the minimum being 42 days, at a temperature of 20°C, and a relative humidity of 100%. Krishnamurthy et al. (1963) found that, at a temperature between 25-28°C, and a relative humidity between 75-80%, the life cycle could be completed in 24-29 days.

The spiracles control the loss of water, according to Mellanby (1934), who found that in adult fleas exposed to 5% CO₂, the rate of water loss is doubled because the spiracles are permanently open. Humidity is somewhat unimportant, then, since the flea at rest keeps most of its spiracles closed (Wigglesworth, 1935).

Buxton (1948) states that the adult flea is hardly affected by humidity, and in its natural habitation the water loss would probably be made up at the next feeding. Smith (1951) found that high humidities cause adult female activity to increase to the extent that the female needs frequent blood meals.

Light sensitivity.--Adults of X. cheopis are photonegative. (Mitzmain, 1910, used the term "negatively heliotropic" while Pausch, 1962, described this condition as "negatively phototactic.") Edney (1945) reported that adults kept in the dark live slightly longer than those in the light. This is true even if the pre-adult stages have been passed in the dark. Smith (1951) states that fleas of the genus Xenopsylla are repelled by strong light, and that they are most active in air which is saturated or nearly so.

Feeding.--There is no difference in size of newly emerged adults which have not fed several times (Edney, 1945). Once adult fleas have

fed, they must continue to do so or else they will die (Hirst, 1923). A flea which has ingested a blood meal usually will not feed again until the following day. The adults are persistent feeders, and will continue feeding even though they are engorged, to the extent that blood is excreted through the anus and falls down to the substrate. This habit (or behavior) is probably stimulated by adenosine triphosphate, which is found in the host's blood (Galun, 1966). The average adult will engorge approximately 0.5 cc of blood at one feeding, according to Reports on Plague Investigations in India (1907). Maximum feeding was obtained with fleas 5-8 days old by Bar-Zeev and Sternberg (1962). It was found that starved females ingest more blood than starved males. Both Mitzmain (1910) and Pausch (1962) say that the female must have a blood meal before copulating or laying eggs.

Fox et al. (1966) found that X. cheopis will feed on invertebrate as well as on vertebrate blood. Placed on a Puerto Rican lizard, Anolis cristatellus cristatellus, the fleas began to feed either immediately or within 5 minutes. The longest life of an adult in the experiment was 36 days, while the minimum was 4 days. Under the conditions of this experiment no progeny was obtained.

Mating.--Suter (1964) described pictorially the copulatory positions of male and female. He reported that before copulation the females need several blood meals; however, this is not true of the males which, in fact, can copulate immediately after emerging from the pupae, one male being able to mate with several females. Transfer of sperm takes place in about 10 minutes. Mating may take place on the host, in the nest, or in the bedding.

Miscellaneous.--Smith (1951) stated that in unfed female fleas up to one day old, there was no daily rhythm of activity.

Shulov and Naor (1964) reported that X. cheopis females from 5-10 days old were attracted more readily to male white rats than at any other age. In fact, for the first 3 days of their experiment the female fleas were repelled.

Reproductive system

Female.--In the adult female an ovary lies on either side of the mid-intestine. Each ovary consists of from 3-6 panoistic ovarioles; that is, ovarioles which lack specialized nutritive cells (Figure 1). (Among the holometabolous insects, the Siphonaptera is the only order which possesses the panoistic type of ovariole, according to Bonhag, 1958.) Normally the germarium in the ovariole contains many oögonia. Here an occasional oögonial cell will undergo mitosis to form young oöcytes. These oöcytes move into the vitellarium or zone of growth where prefollicular tissue surrounds them. This tissue then becomes the follicular epithelium of the primary oöcyte. The primary oöcytes, then, are separated from each other by an interfollicular zone of tissue. The oöcyte nucleus is called the germinal vesicle. Surrounding the ovariole is a thin outer epithelial sheath. Each ovariole has a terminal filament which unites to form the ligament of the ovary. The ligament is attached to the pericardial diaphragm.

The lateral oviducts from each ovary unite to form the common oviduct which opens at the vagina located between the eighth and ninth abdominal sternites. The spermatheca lies below the rectum, and the spermathecal duct opens into the front wall of the vagina.

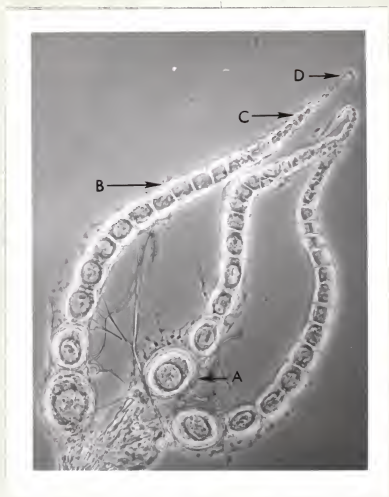


Fig. 1.--Female ovarioles showing primary oöcyte (A); oöcytes in various stages of development (B); germinal region (C); and terminal filament (D). 130x.

In normal circumstances, the flea has only one spermatheca, but Sharma and Joshi (1961) have reported an adult with two. The extra one was pear-shaped with no distinct head portion, whereas the other was of the normal hook or sickle shape.

Male.--The adult male has one pair of oval-shaped testes which have one layer of epithelial tissue (Wasserburger, 1961). At the base of each testis is a cup-like structure which contains a coiled duct or epididymis (Gunther, 1961). From this coiled duct comes the vas deferens which enters the seminal vesicle (Patton and Evans, 1929) or ejaculatory bulb (Gunther, 1961). It then enters the ductus ejaculatoris from which it proceeds to the aedoeagus. There are two pairs of accessory glands (one short and one long), which unite with the vas deferens near the testes (Figure 2).

The egg

The egg of X. cheopis is oval, and when newly laid it is pearly white in color and slightly translucent. It is viscous, and adheres to the substrate (Mitzmain, 1910). The length is from 0.450 mm to 0.475 mm, and the width is 0.3 mm (Bacot and Ridewood, 1914, and Webster, 1929). There are 56 micropylar openings at the anterior end and 32 at the posterior end (Cameron, 1939).

Egg production

Egg production in the female seems to vary. Patton and Evans (1929) state that the female may lay from 2-6 or more eggs at a time, and that she is capable of laying from 300-400 eggs during a lifetime. Mitzmain (1910) reported that the majority of eggs obtained were laid on the first day, and that the average number was 6. In 1908, the

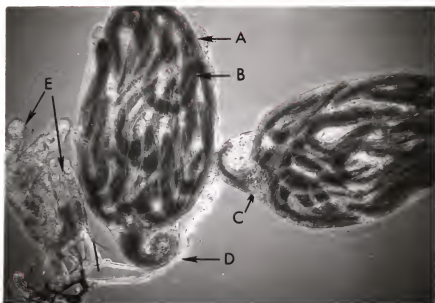


Fig. 2.--Male. (A) testis; (B) sperm; (C) epididymis; (D) vas deferens; (E) paired accessory glands. 111x.

Report on Plague Investigations in India reported that from 1-5 eggs at a time are laid, one after the other; however, another of these reports (1912) indicated that the average number laid per wild flea in monthly periods for two and one half years ranged from 0.6-3.0. Their figure was based, however, on only one hour of egg-laying time. This report also states that the largest number of eggs was laid during the first hour after separation from the rat hosts.

In 1955, Hopkins reported that with a "perch" (resting material) the average egg production per laying female was 3.8, while without a "perch" it was 4.6. Pausch (1962) obtained an average of 2.2 eggs per fed female in his experiments.

Buxton (1948) found that fleas produced an abnormally low number of eggs when fed on mice 9-11 days old; however, they laid more eggs when allowed to feed on adult mice for several hours before being placed on baby mice. He was of the opinion that the younger mice lack a sex hormone which stimulates egg production in the female flea. Rothschild, however, stated in 1965 that X. cheopis is not dependent on rat sex hormones. If the rat has been castrated, and its adrenal and pituitary glands removed, the flea fed upon it will still copulate and lay eggs, although it is not known if the eggs will produce progeny.

Buxton also found that higher temperature produces earlier egg-laying and a greater quantity of progeny per day, and that progeny from parents fed on the adult mouse emerge sooner than those fed on the baby mouse.

Longevity

Burroughs (1953) discovered that if X. cheopis adults were fed daily on a guinea pig, they lived longer than those fed only once or not at all. One male flea survived for 72 days; the maximum survival time was 158 days for a female which had had daily access to a rat. Burroughs concluded that:

The opportunity to obtain frequent blood meals had the greatest effect of any single factor in producing individual long-lived fleas.

This is also the opinion expressed by Leeson in 1936.

It is interesting to note that Bacot stated in 1914 that X. cheopis may live for up to 10 months.

In 1933, Hirst made reference to the fact that fleas held in glass tubes should have some type of resting material. Hopkins (1935) conducted tests to determine if the use of resting material would prolong the life of the flea, and whether or not it was conducive to egg-laying. He found that without a "perch" the average life for the male was 5.6 days and for the female 9.9 days. With a "perch," the average was 6.2 days for the male and 7.1 days for the female.

Some wild-caught fleas, anesthetized with chloroform, had a shorter mean longevity than did those not anesthetized. Crowding of larvae, which reduces adult longevity, also causes a reduction in the size of the adults (Edney, 1947b).

Longevity of F_1 adults is affected by the age of the parent fleas (Edney, 1947b). Those from parents less than 10 days old live longer than those from parents more than 11 days old.

According to Burroughs (1953), adults which have continuous access to blood meals will live longer than unfed individuals. This is the

opposite of Edney's conclusion (1947b) that:

Well developed, unfed, laboratory-bred adult fleas live longer than those which have fed once before starvation, and the latter live longer than those which have fed several times.

X. cheopis, unlike many other parasitic insects, does not need to remain upon its host. A part of the adult life is spent in the nest of the host.

The larva

The larva hatches from the egg in from 2-10 days, depending on the temperature (Bacot and Ridewood, 1914, and Patton and Evans, 1929). The external morphology of the larval instars has been fully described by Bacot and Ridewood (1914), Henderson (1928), and Sikes (1930). The following general account of the larval stage is condensed primarily from writings of Bacot (1914), Bacot and Ridewood (1914), and Sikes (1930).

The newly hatched larva emerges from the egg with the aid of a hatching spine or egg-breaker which is located on the epicranium. It is about 1.4-2.0 mm long, cylindrical, semi-transparent, and about 0.22 mm thick. Living upon almost any kind of refuse, animal or vegetable, this larva will even feed on drops of dried blood which the adult flea has defecated.

It undergoes two molts during the active stage, and one in the cocoon. While molting, it will lie straight, except in the third molt when it is bent to form a U-shape. In most instances the larva is very active; however, there are times when it will become quiescent and lie coiled up.

When the temperature is between 25-28°C, and the relative humidity between 75-80%, the larval period is from 13-15 days (Krishnamurthy et al., 1963).

The rearing media should not be wet, but the humidity should be between 75-90% for best larval growth. In 1949, Sharif reported that for the larva the critical humidity varies with the temperature. He demonstrated (1948a) that the chief source of water gain in the larva is through its food, and further concluded that the water requirement is slightly less for the male than for the female larva.

The nutritional requirements for the larva are simple according to Sharif (1948b). It can even live in the domestic rat burrow where the nutritive value of larval food is low. Pausch (1962) readily grew larvae on an artificial diet of 100 parts vitamin-free casein, 2 parts Wesson salt mixture, 1 part cholesterol and 9 vitamins.

Edney (1947b) reported that if too many larvae are reared together, even though there is sufficient food, the adult longevity is reduced. He also found that if larvae are reared in fine rather than coarse sand, the adults are unable to develop to their normal size. The fine sand abrades the larval epicuticle, causing water evaporation which cannot be regained even in high humidity.

Prepupa and pupa

Prepupa.--Hopkins (1935) reported that the female has a shorter larval period than the male. The fully grown larva is creamy white in color, and the alimentary canal is devoid of food. It then spins a cocoon of silk and debris. It is active at first, and any sudden stimulus or rise in temperature will cause it to leave the cocoon (Mellanby, 1933). Under normal circumstances the larva soon ceases

movement, and remains folded double within the cocoon. This is the prepupal stage.

If kept at a temperature of 24°C and a relative humidity of from 50-90%, the prepupa will gain weight by the absorption of water vapor from the air (Edney, 1947a). A relative humidity below 45% will kill it. The prepupa molts to the pupal stage in about 3.5 days.

Pupa.--Under the rearing conditions presented by Krishnamurthy et al. (1963), the pupal stage was from 6-8 days. Mellanby (1933) stated that, although the pupa is resistant to dry air, the prepupa is not, so that it is necessary to maintain a humidity of at least 60% or higher, along with a temperature of between 18-35°C.

Normally the pupal period of the female is shorter than that of the male (Hopkins, 1935). Edney (1947a) found that adults which have emerged from pupae kept at higher humidities weigh more, have a higher percentage of water content, and live longer without food than those emerging from pupae kept at lower humidities.

Sharif (1935) discovered that the pupae of the fleas Nosopsyllus fasciatus (Bosc), Leptopsylla segnis (Schonherr), and Ceratophyllus gallinae (Schrank) have wing buds on the mesothorax; he could find none on X. cheopis.

METHODS AND MATERIALS

Rearing of *X. cheopis*

The Orlando strain of *X. cheopis* was used in all experiments. The fleas were mass-reared according to the methods of Smith and Eddy (1954) and of Gilbert (1964). Approximately 500 newly emerged adult fleas were put on a caged rat in a rearing pan containing approximately 1.5 liters of dry sand and 100 cc of powdered dog food. The rat was fed daily with pieces of dog food and apple. Approximately 21 days later it was removed, and the contents of the pan sifted through 8- and 16-mesh screens to remove debris and cocoons. The sand containing eggs, larvae, and adults was then placed in an enamel pan and covered with organdy cloth. This pan was placed in an incubator where the temperature was $28 \pm 2^{\circ}\text{C}$, and the relative humidity was $80 \pm 5\%$. The sand was sifted through the 16-mesh screen once a week for 3 weeks in order to obtain the cocoons.

Cytological techniques

Studies were undertaken to determine the effects of the chemosterilant tepa on flea chromosomes. Before proceeding with these studies, however, it was necessary to determine what tissue of the flea contained the most mitotic chromosomes, and at which stage of the flea's development. Many experiments proved that the best object for these studies was the supraoesophageal ganglion of the prepupa.

Under a dissecting microscope at 30X, the prepupa was dissected out of the cocoon with two pairs of extra-fine forceps in the modified Ringer's solution of Morgan and LaBrecque (1964a). The prepupa was then transferred to a deep-well slide containing Ringer's solution. The prothorax was held firmly with one pair of forceps while the head capsule was gently pulled away with the second pair. The supraoesophageal ganglion, easily observed as two white oblong lobes attached to the prothoracic ganglion, was then severed from the ganglion with the forceps, and transferred for 10 minutes to a 1.0% hypotonic solution of sodium citrate. After being placed in modified Carnoy's fixative for 5 minutes, it was removed to a small drop of 45% acetic acid on a clean microscope slide which had been washed in acid alcohol. The tissue was covered with a siliconized cover slip.

Placed between the folds of a piece of filter paper (150 mm), the covered slide was then gently but firmly tapped over the tissue area 20 or 30 times with a moderately heavy rod. After this, the slide, still wrapped in filter paper, was squashed with a chromosome squash apparatus (Linkfield et al., in press), after which it could be quickly scanned under the phase microscope for mitotic divisions.

The chromosome squash apparatus is based on the fulcrum principle, the pressure being supplied by a 25-pound lead weight. The slide containing the tissue to be squashed is placed under the pressure plate within a folded piece of filter paper. Using the apparatus at 166 pounds of force (216 lbs. psi for a 7/8" x 7/8" cover slip), excellent preparations were made.

The slide at this stage was placed on a piece of dry ice for a minimum of 30 minutes. Staining and permanent mounts were then made according to the technique of Morgan and LaBrecque (1964a).

To determine what cytogenetic effects tepa has on the somatic chromosomes, a test was initiated using 20 larvae which were ready to pupate. A filter paper with a diameter of 9 cm was treated with tepa at 10 mg/cm^2 , and allowed to dry for about 16 hours. The larvae were placed on the treated filter paper for 4 hours. They were then removed to untreated paper for a 24-hour period. After this time, the supraesophageal ganglions of several larvae were prepared according to the procedures previously described.

Experimental design

(To achieve maximum readability, the word "treat" in all its parts of speech is used only in reference to the use of the chemosterilant.)

Male laboratory white mice from 30-40 days old were used as hosts in all experiments.

A preliminary test showed the following procedures to be the most expedient for all experiments.

Flea populations for experimental use.---Prior to the treatments, newly emerged adult fleas were collected in a one-quart Mason jar containing a 9 cm filter paper upon which the fleas could rest. A piece of black organdy cloth was placed over the mouth of the jar before the rim was screwed on. The fleas were kept in the dark until they could be sexed, which was usually within 24 hours. Ether, U.S.P., was poured into a screw-top, ten-dram glass vial which contained a crumpled sheet of Kimwipe^R paper, type 900-S. When not in use, the vial was kept in an explosion-proof refrigerator.

On the second day, the adult fleas were sexed. The glass vial containing ether was slowly lowered into the Mason jar containing fleas, and the jar lid was screwed on tight until all activity had ceased,

after which time the vial was removed. The anesthetized fleas remained inactive for about 10 minutes. Approximately 30 fleas were poured into a plastic petri plate top which was then placed under a zoom binocular microscope and viewed at 10X. A suction apparatus (Fig. 3) was used to pick up the fleas. Male and female fleas were put into separate one-quart Mason jars, each of which had a 9 cm filter paper placed on the bottom. The adult fleas were held in the dark for recovery from the anesthetic.

Chemosterilant treatment.--Whenever chemosterilant treatments were being conducted, rubber gloves were worn, and treatments were made in a negative-air-flow hood.

Filter paper strips (Fig. 4) used in all experiments were 7.3 cm^2 , similar to those reported in the World Health Organization Technical Report of 1963. Before treatment, the paper strips were securely stuck to a pinning board (Fig. 4). A small galvanized enamel pan was partially filled with cider vinegar (or 3% acetic acid), and a vial containing the chemosterilant, tepa dissolved in methanol was set in the pan. A pro-pipet was fitted to a 1 ml pipet, and 0.13-0.16 ml of the chemosterilant solution was applied to the filter paper strips at a dosage of either 5, 10, or 15 mg/cm^2 .

With a clean pipet, 0.13-0.16 ml of methanol was applied to all control strips. Both groups of strips remained in the hood for 24 hours to dry.

After treatment of strips, cider vinegar was poured into the chemosterilant vial in order to deactivate the remaining active material before the vial was discarded. The pipet and all other reusable materials which came in contact with the chemosterilant were



Fig. 3.--Vacuum aspirator used to sex adult fleas.

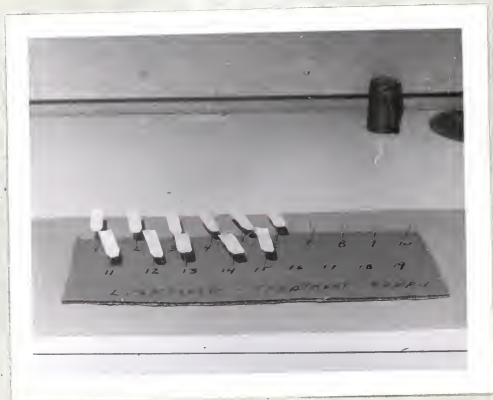


Fig. 4.--Pinning board used for treating filter paper strips.

thoroughly soaked in cider vinegar for 5 minutes before being washed in soapy water.

The treated filter papers were picked up with a pair of forceps, and placed in 18 x 150 mm test tubes. Twenty fleas were placed in each of 5 tubes, the open ends of which were covered with a piece of white organdy cloth held in place by a cardboard collar. Depending on the type of reciprocal cross experiments to be conducted, either males or females were treated with the tepa. The fleas were held in the tubes for varying lengths of time (2, 4, or 6 hours).

After the required treatment time, the treated fleas were transferred to post-treatment test tubes each of which contained a clean filter paper strip. Controls were transferred to like tubes at the same time. The open end of the post-treatment test tube was placed below the treated one at an angle of about 40 degrees. The treated tube was gently tapped until the fleas fell into the clean tube. If the treated filter paper started to slide toward the clean tube, the tubes were separated and held upright while the treated paper resettled on the bottom, after which the procedure was repeated until all fleas were in the post-treatment tube. Sometimes a few fleas could not be dislodged from the paper strips by this method; however, when the tubes were held close to a light, the fleas would begin to move, at which time they would be tapped into the clean tube. These steps were taken in order to avoid contamination of an aspirator.

Contaminated test tubes and filter paper strips were handled as previously described for contaminated material.

Sterility experiments.--Treated adult fleas were held in the dark for 24 hours, after which they were checked for mortality. Those not dead were used for determining which dosage effected sterility.

In initial sterility experiments, 5 male and 5 female fleas were used, but in subsequent testings it was decided to use 10 fleas of each sex. In order to demonstrate sterility, the following reciprocal crosses were made:

1. Untreated females X Untreated males
2. Untreated females X Treated males
3. Treated females X Untreated males
4. Treated females X Treated males

Mice confined in small, round, wire cages were lowered into one-quart Mason jars with an unwound paper clip of the larger size, bent to hook at one end. The bottom of each jar contained a 9 cm filter paper, along with a strip of blotter paper to absorb the mouse's urine.

An equal number of both sexes of fleas was put into a labelled Mason jar with the caged mouse. The jar was then placed in the larger side of a double-compartment Vacucl^R ice chest (Fig. 5). About two quarts of water were poured into the smaller compartment. Black organdy cloth was draped over the top of the ice chest in order to maintain darkness and as much humidity as possible.

The caged mouse was removed from the Mason jar daily, fed, placed in a freshly prepared jar, and returned to the chest. It should be noted that, when feeding the mouse, care had to be taken not to lose fleas. The mouse cage was picked up with the paper clip hook in one hand, grasped with the fingers of the other hand, and quickly transferred to the new jar, with food being given to the mouse through a hole in one end of the cage before it was completely lowered into the jar. In most instances the fleas were around the tail area of the mouse, but a bulb-aspirator was kept at hand when making transfers in case a flea would jump off.



Fig. 5.--Vacucler^R ice chest used to maintain darkness and humidity for experimental mice and fleas in Mason jars. (A) black organdy cloth; (B) water compartment; (C) compartment for Mason jars.

The primary reason for placing the mice in cages was to prevent them from eating the adult fleas, as was reported by Leeson (1936). Cameron (1939) stated that when fleas were first introduced upon a healthy mouse, it ate many of them.

The used Mason jars were taken to the binocular microscope to look for flea eggs. It was found best to remove the papers from the experimental jar before aspirating out any fleas to be transferred to the clean jars. In order to position the blotter and filter papers for removal from the jar, the paper clip hook was used, with extreme care being taken not to damage the eggs.

The blotter paper was removed with a pair of forceps and placed in a plastic petri dish cover 100 x 15 mm. It was then scanned under the binocular microscope at 10X. If eggs were found, a drop of modified Ringer's solution was put on the paper close to the egg, softening the paper and allowing the egg to be picked up with ease by the use of fine needle-nosed forceps. The egg is less likely to be damaged by this method, as some of the paper is removed with it.

After the top of the blotter paper had been scanned, the reverse side was checked. This same procedure was carried out on the filter paper, and on all droppings and debris left in the bottom of the jar.

The flea eggs were placed on a 3.8 cm black organdy cloth disc in a plastic petri dish, which was then set in an incubator (Fig. 6). The incubator was kept at $26 \pm 2^{\circ}\text{C}$, with a relative humidity of $86 \pm 2\%$. A whisper fan was installed to provide air circulation, and the humidity was maintained with a sponge partially immersed in distilled water in a plastic food container. The entire humidity unit was changed weekly, or sooner if necessary. The temperature and humidity were checked daily



Fig. 6.--Incubator. (A) whisper fan; (B) sponge and water container for maintaining humidity; (C) petri dishes containing eggs and growing larvae; (D) temperature-humidity probe; (E) temperature-humidity recorder.

with the aid of an electric hygrometer indicator (Hygrodynamics, Inc.). The temperature-humidity probe remained in the incubator throughout the testing period.

The eggs were checked daily for hatch for 6 days. Larvae were picked up with a very fine paint brush (#00), and placed in a dated plastic petri plate containing sand and ground-up rat chow. The petri plate, marked with the date on which the eggs were laid, was placed in the incubator. At the beginning of the third week an emergence chamber was placed over the rearing media so that emerging adults could not escape.

The emergence chamber.--The bottom was punched out of a one-pint cardboard ice cream container, and the top ridge trimmed off with a razor blade. The unridged top was then gently inserted into the petri dish containing larval medium and maturing larvae. The container was fastened to the petri plate with cellophane tape, and organdy cloth was secured to the top with a rubber band. These containers made ideal adult emergence chambers (Fig. 7). When adults began to emerge, the organdy cloth was removed and they were aspirated out of the chamber each day.

Statistics.--The negative binomial transformation of Bartlett (1947) was used to stabilize the variance of the original egg production data. The pooled "t" test of Snedecor (1962) was then used to test the difference in egg production between first and second weeks of control and of chemosterilant experiments.

The paired "Z" test of Mendenhall (1964) was used to determine the difference in emergence of larvae and F_1 adults in control and chemosterilant experiments. Percent sterility was figured according to the method of Crystal (1963).



Fig. 7.--Adult emergence chambers

The predicted egg production curve was calculated using the formula $y = K(1 - e^{-rt})$ where y = egg production; K = asymptotic constant; $e = 2.7183$; r = growth constant; and t = time.

Photography.--Photomicrographs were made through an American Optical Phasestar microscope with Ortho-illuminator. They were taken on 135 mm Panatomic X, Black and White film (ASA 32), and the green filter of the Ortho-illuminator was used.

RESULTS AND DISCUSSION

Biological Observations

Ovarioles

Wasserburger (1961) stated that he regularly found 5 ovarioles in each ovary of the female X. cheopis and Patton and Evans (1929) 4-6. While measuring the ovarioles and testes of the Orlando strain of the flea (Table 1), this author found ovaries with 3, 4, and 6 ovarioles each. Although none was found with 5 ovarioles, they could very well have been present in the Orlando strain. The variation in ovariole number could explain why there was some difference in egg production from one test to another, especially in one control test. There was no difference in overall egg production between the first and second weeks of 12 experiments ($t=1.657 < t_{.05}=1.771$).

Egg production

Figure 8 shows the daily egg production per female for 14 days, based on the average number of eggs counted daily from 120 females. Apparently all the primary oöcytes are not mature at initial egg-laying. After about 4 days the ovarioles become synchronized and egg production increases probably close to its maximum. The mean daily egg production per female was $4.25 \pm .21$.

A growth curve (Fig. 8) was calculated using the least squares method. It was based on the egg production per day in 12 experiments. The predicted daily egg production constant was 4.75 eggs per female.

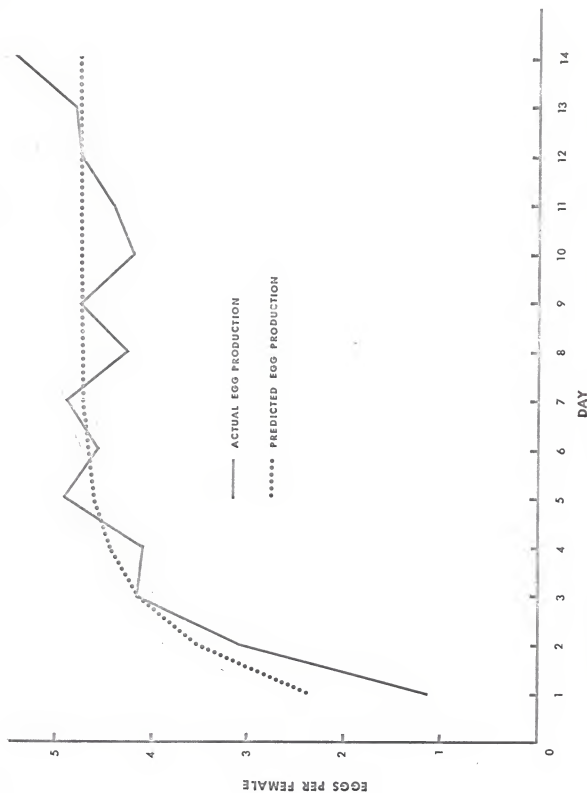


Fig. 8.--Average and predicted daily egg production per X. cheopis female from 12 experiments of 14 days each.

Table 1.--Measurements of gonads of 30 male and 30 female
X. cheopis adults.

Dimension	Mean in mm	Range in mm	Standard Deviation
MALE			
Length of:			
Right testis	.62	.53-.70	.014
Left testis	.63	.49-.70	.020
Width of:			
Right testis	.34	.32-.39	.007
Left testis	.33	.28-.35	.007
FEMALE			
Length of:			
Ovariole	.66	.53-.77	.015
Width of:			
Primary oöcyte	.10	.07-.14	.008

The egg production per female from 10 females in a control experiment is shown in Fig. 9. This experiment, which was continued for double the initial 14 days, showed a higher egg production than any other. The increase is possibly due to the fact that almost all of these happened to be females with more ovarioles than the other experimental females. Nevertheless, the data are valid and informative.

It is interesting to note that the highest daily egg production was 12.6 eggs per female. These females laid 80 or more eggs on 15 different days, while less than 80 eggs were laid on the other 13 days.

Ratio of male to female

The ratio of male to female adult fleas is very near 1:1. Bacot et al. (1914) proposed that in nature the females of X. cheopis are in excess of males by 15.05%. Their specimens were collected from host animals in the field. As can be seen from Table 2, their assumption is borne out in a calculation of the sex ratio in 2 of the author's experiments. Here all fleas were sexed and counted, and females were found to be in excess of males by 13.66%. Apparently males remain on the host as often as do the females; during observations by this author, male fleas were found mainly on the host. The only time they were seen off the host was when they were with some of the females on the filter paper.

Behavior

According to Wigglesworth (1964), the hormonal control of reproduction might be stimulated by the act of copulation, as in the cockroach, Diploptera punctata. In this species, mating appears to be needed for the normal rate of egg growth. This might also be true of X. cheopis.

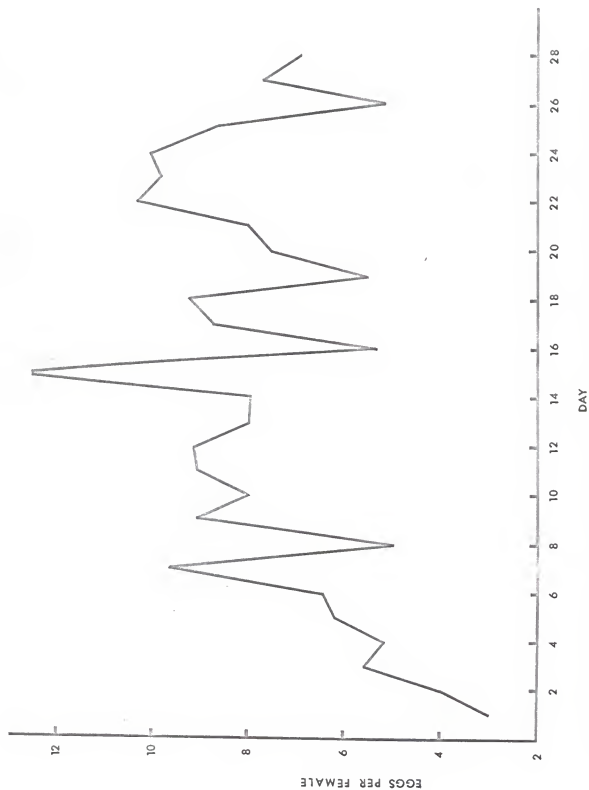


Fig. 9.--Average egg production per female for 28 days based on that of 10 females in one control experiment--

Table 2.--Ratio of female to male in laboratory-reared and in field-collected X. oregonis adult populations.

PERCENTAGE OF		
Females	Males	Excess females over males
Laboratory-reared ^a		
53.20	46.80	13.66
Field-collected ^b		
53.50	46.50	15.05

^a735 emerging F_1 adults in 2 of the author's laboratory experiments.

^b8,489 fleas collected in several Egyptian locales, and examined by Bacot et al. (1914).

Norris (1954) found that the female Schistocerca gregaria must copulate in order to lay her full complement of eggs. Bacot (1914) reported that in his experiments no X. cheopis female laid any eggs without having fed first. He also stated that oviposition has always been preceded by several days of feeding.

Copulation, however, does not appear to be necessary for egg-laying (Fig. 10). The stimulating factor for both copulation and egg-laying, then must be blood meals. Females which have not had a blood meal will neither copulate nor lay eggs. Nevertheless, virgin females will lay eggs at least for a period of 14 days, and probably for longer if they have access to the necessary blood meals. The number of eggs produced decreases with time, but does not appear to cease completely.

There seems to be a difference in egg-laying behavior between mated and virgin females. Mated females will lay their eggs in clusters, or sometimes singularly, on or underneath the filter paper, on mouse feces, or on bits of rat chow. In most instances these eggs will have bits of rat chow attached to them (Fig. 11). They are difficult to see (Fig. 12) without correct lighting. The surface of the egg has a sticky substance which, when dry, adheres the egg to the substrate, and bits of rat chow to the egg.

In view of the preceding observations, it is difficult not to believe that the female fleas have actually placed bits of the rat chow around the newly laid eggs, as many of these covered eggs can be found several inches from the main area of debris. Even if they had fallen from the mouse onto the rat chow bits, it is unlikely that they could have rolled so far away and almost impossible for them to have adhered

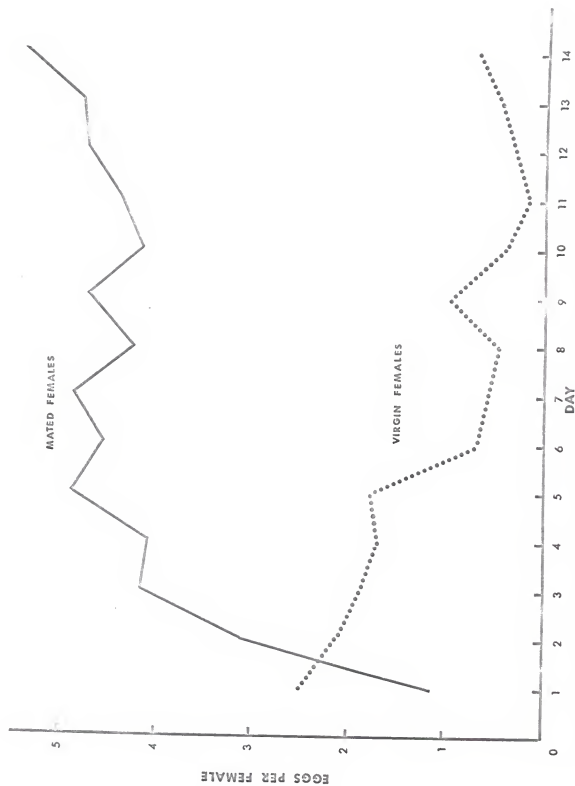


Fig. 10.--Average egg production of mated and of virgin X. cheopis females which had continuous access to blood meals.



Fig. 11.--Bottom view of egg of X. cheopis adhering to bits of rat chow. 44x.



Fig. 12.--Top view of *X. cheopis* egg (A) which is stuck to the filter paper, and surrounded by bits of rat chow. 44x.

to the filter paper. Closer observation of egg-laying, and further research into this probable behavior, would be most interesting.

Eggs from virgin females do not have this sticky substance, since their eggs can easily be removed from the substrate with a dry paint brush or with forceps. No virgin females were observed off of the host even though mated females were often seen on or under the filter paper.

Copious feeding causes extrusion of blood from the anus, providing nourishment for the larvae. The placing of food around the egg could be a behavior pattern for providing food for the emerging larvae, in which case the virgin female is unable to provide it. This behavior, as well as the production of the sticky substance, could be caused by stimulation of the female collateral glands during copulation. There is also the possibility that the male inseminates a substance during copulation which stimulates the female to initiate these behavior patterns.

Parthenogenesis

According to White (1964) there appears to be no literature regarding parthenogenesis in the Siphonaptera (Aphaniptera) and in five other orders of insects.

In the virgin female egg production experiment, it is interesting to note that of 149 eggs produced in 14 days, 4 underwent partial embryonic development (Table 3). In one egg the head capsule was easily found, while in the others only setae were observed. These embryos did not hatch. Suomalainen (1962) called this rudimentary parthenogenesis; that is, when an unfertilized egg from a bisexual species starts to develop, it can be expected eventually to stop

Table 3.--Production and development of eggs from virgin female X. cheopis which had continuous access to blood meals.

Day ^a	Eggs Produced	Embryos Formed	Hatch
1	25	0	0
2	21	0	0
3	19	0	0
4	17	1	0
5	18	1	0
6	7	0	0
7	6	0	0
8	5	1	0
9	10	1	0
10	4	0	0
11	2	0	0
12	3	0	0
13	5	0	0
14	7	0	0
Totals:	149	4	0

^aEggs were first observed on the 3rd day after placing fleas on host.

development and die. Sometimes, however, an unfertilized egg will hatch and produce an adult; this is called tycho parthenogenesis (accidental parthenogenesis). In certain species of *Drosophila* the rate of parthenogenetically developing adults was 2/37,628 and 1/19,059 (Suomalainen, 1962).

Since it has been shown that embryonic development can take place in eggs laid by virgin X. cheopis females, further experiments into the possibilities of tycho parthenogenesis should be initiated.

General observations

Life cycle.--The author, under the conditions of his experiments, found that the life cycle could be accomplished in 15 to 21 days at a temperature of $26 \pm 2^\circ\text{C}$, and a relative humidity of $86 \pm 2\%$. By 28 days emergence was complete. Each experiment took approximately 6 weeks to complete.

Larval and F_1 adult emergence.--Figure 13 shows the daily percent total number of eggs deposited, and larval and F_1 adult emergence from the 4 control experiments. The total larval emergence was 81.09%, while the F_1 adult emergence was 90.12%.

Feeding site.--Bacot (1914) stated that rat fleas pick a special "point of vantage, X. cheopis making for the shoulders, neck and chest or for a spot beneath the forelegs." This author, however, observed that the fleas in all his experiments were usually to be found around the tail and scrotal area of the caged mouse. In fact, newly introduced fleas went directly to the posterior end. Additional studies would no doubt show whether or not this is the preferred feeding site. It is possible that, since mice usually clean themselves quite well around these areas, they eat many of their pests found there, and this is why

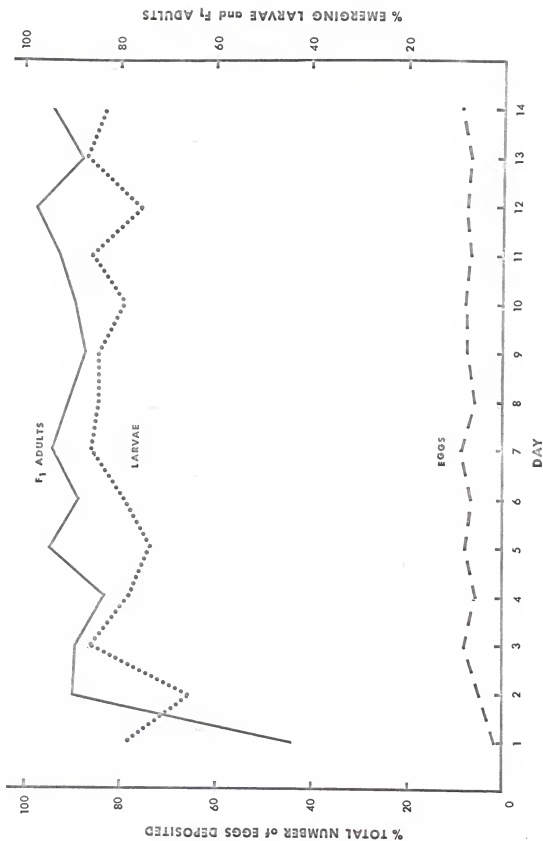


Fig. 13.--Rate of egg-laying and percent emergence of larvae and F_1 adults.

fleas on uncaged mice were found in the areas mentioned by Bacot, even though they prefer the other.

Chemosterilization with Tapa

Egg hatch

Eggs from female fleas which had been either treated, or crossed with a treated male, were retained for 6 days, after which, if there had been no larval emergence, they were discarded. The control larvae usually had emerged by the third day. Data on egg-laying and on larval and F_1 adult emergence for all of the chemosterilant experiments and for the controls are summarized in Table 4. Tables 5 through 24 in the Appendix contain the results of each individual experiment.

Natural sterility

The natural sterility obtained in 4 laboratory control experiments was 18.45% in the larvae and 10.14% in the F_1 adults.

Egg breakage

In an initial sterility experiment many eggs were broken in an effort to remove them from the filter paper. Since 34 eggs from the control alone were broken in the first 8 days, it was decided to try Ringer's solution in order to soften the filter paper and allow easier removal of eggs. On days 9-14 the solution was used. Only 6 eggs were broken during this 6-day period. It appeared that with Ringer's solution egg breakage would be reduced considerably and larval hatch improved. Therefore a statistical analysis using the paired "Z" test was made. Since Ringer's solution was found by this analysis to improve hatch ($Z=2.833 > Z_{.05}=1.645$), it was used to remove eggs from the filter paper in all experiments.

Table 4.—Summary of results from experiments using tepa in varying dosages and lengths of time for sterilization of *X. cheiridis* adults. Ten males and 10 females were used in each 14 day experiment.

Treatment	Dosage mg/cm ²	Contact Time hours	RANGE											% Sterility Based on Emergence of
			AVERAGE NUMBER OF											
			Eggs		Emerging		Eggs		Emerging		Larvae			
			Produced	Survived ^a	Larvae	F ₁ Adults	Produced	Survived ^a	Larvae	F ₁ Adults	Larvae	F ₁ Adults		
Treated female X untreated male:														
5		4	3-45	3-45	1-38	0-21	22.00	21.00	16.00	8.00	69.20	84.00		
5		6	4-24	4-24	0-14	0-11	6.00	6.00	4.00	2.00	98.20	99.28		
10		4 ^b	3-12	3-11	0-1	0-0	0.60	0.60	0.07	0.00	99.99	100.00		
Treated male X untreated female:														
5		6 ^b	12-64	12-64	0-30	0-0.33	44.00	43.00	0.2	0.05	99.00	99.99		
10		4	6-60	6-57	0-9.00	0-4.00	39.00	37.00	4.0	0.80	85.30	99.50		
10		6 ^b	13-59	13-59	0-1.30	0-0.67	42.00	42.00	0.2	0.10	99.20	99.99 ^c		
15		6	5-50	5-50	0-2.00	— ^c	32.00	32.00	0.1	— ^c	99.99	— ^c		
Treated female (F) X treated male (M):														
5 (F)		6	0-5	1-4	0	0	0.70	0.60	0	0	100.00	100.00		
10 (M)		4												
Untreated female X untreated male:														
CONTROL ^d			13-58	12-56	10-43	5-45	46.00	44.00	36.00	32.00	13.46	10.14		

^aSome eggs were found broken, or were broken in handling.

^bAverage of three replicates.

^cExperiment was terminated 6 days after Day 14 because 100% sterility had not been achieved, and treated male mortality was 10% in the 24-hour post-treatment period.

^dAverage of four replicates.

In the first hour after oviposition, the zygote is formed in the egg (Cameron, 1939). For the first 8 days (no Ringer's solution) the larval hatch was only 60%, while for the remaining 6 days (when the solution was used) the larval hatch was 73.60%. It is possible that maturation of the egg nucleus and fusion of the pronuclei had been disrupted during removal from the filter paper without Ringer's solution.

Female sterilization

5 mg/cm².--Ten females treated with tepa at 5 mg/cm² for 4 hours and mated with untreated males, had a decrease in egg production the first week ($t=3.53 > t_{.05}=2.447$), while in the second week there was no significant difference between egg production of the control fleas and of those treated ($t=2.39 < t_{.05}=2.417$). Tepa significantly reduced larval hatch in the first week ($Z=33.85 > Z_{.05}=1.645$), while in the second week there was little difference ($Z=0.372 < Z_{.05}=1.645$). The emergence of the F_1 adult generation, however, was significantly reduced in both the first and second weeks ($Z=5.556 > Z_{.05}=1.645$ in the first week; $Z=4.560 > Z_{.05}=1.645$ in the second). The larval sterility was calculated to be 69.20% and the F_1 adult sterility, 84.00%.

As is obvious from these analyses, it is more accurate to base conclusions on the F_1 generation rather than simply on larval hatch. Therefore, the criterion for sterility used in all of these studies was the emergence of F_1 adults.

Bertram (1963) reported that thio-tepa (tris(1-aziridinyl)phosphine sulfide) caused reduced fertility and fecundity in female A. aegypti. Rai (1964) stated that eggs from female A. aegypti treated with apholate must carry induced dominant lethals, since a low hatch was obtained. This is probably true of tepa-treated X. cheopis females as well.

Twenty females treated with tepa at 5 mg/cm^2 for 6 hours, and mated with 20 untreated males, produced some eggs. In one of the experiments 10 females laid eggs for the first 2 days, after which time they did not lay again until the eighth day. Apparently, the primary oöcytes were not affected by the sterilant, but the secondary oöcytes were. At this dosage and contact time, it seems that the germarial region of the ovarioles was not affected, since they did begin to lay eggs again. These females were mated with untreated males, and larval hatch was observed from eggs laid on all days except the first.

In this experiment no F_1 adults were produced from larvae which emerged on the second and the tenth days. Nevertheless, F_1 adults did emerge from the eleventh through the fourteenth days of larval hatch. The larval sterility was 98.20%, and the F_1 adult sterility was 99.28%.

10 mg/cm².--Murray and Bickley (1964) had noted that vacuoles occurred in the ovaries of Culex p. quinquefasciatus when they were treated with apholate in concentrations of 15 ppm and higher.

The female X. cheopis can be completely sterilized (100%) with tepa, using a dosage of 10 mg/cm^2 for a contact treatment time of 4 hours (Table 4). As can be seen in Fig. 14, the ovarioles are completely destroyed. The remnants of the ovarioles are leathery, opaque, and white in color, and are vacuolated. These females had mated with untreated males; Fig. 15 shows a spermatheca with motile sperm in the head.

Male sterilization

In the treated males, 100% sterility was not obtained, although the dosage and treatment contact times were greater than those of the females. Nevertheless, 99.99% sterility was achieved (Table 4).

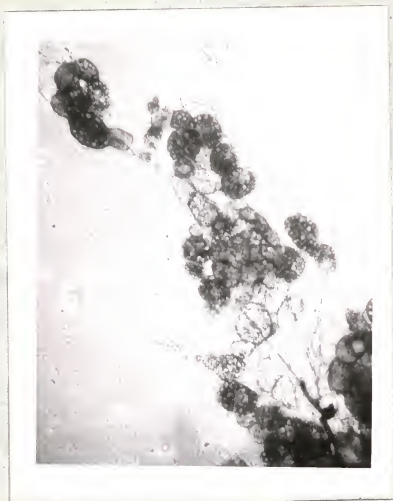


Fig. 14.--Ovarioles of fleas treated with tepa at 10 mg/cm² for 4 hours. Note destruction of ovarioles, and vacuolation. 130x.



Fig. 15.--Spermatheca of a female flea treated with tepa at 10 mg/cm^2 for 4 hours. Spermatheca head (A) contains motile sperm. 120x.

The fact that the males were almost completely sterilized did not affect the egg production of the untreated females; larval hatch and F_1 emergence, however, were greatly reduced.

5 mg/cm².--In an early experiment, 5 males were treated with tepa at 5 mg/cm² for 6 hours, and mated with 5 untreated females. There was no significant difference in egg production between the control and treated for the 2-week experiment ($t=0.0318 < t_{.05}=2.417$ for the first week; $t=1.07 < t_{.05}=2.417$ for the second). The larval sterility, however, was 99.00%, and the F_1 adult sterility was 99.99%.

Since the preceding test gave almost 100% sterility at a low dosage, further tests were conducted at higher dosages to determine the 100% sterility level.

10 mg/cm².--The next test involved the treatment of 10 males with tepa at 10 mg/cm² for a contact treatment time of 4 hours. The males were mated with 10 untreated females. It was reasoned that at a higher dosage, even though the contact time was only 4 hours, 100% sterility could be achieved. This was not the result, however, as is shown in Table 4. In this case the larval sterility was only 85.30% and the F_1 adult sterility was 99.50%.

In another experiment (3 replicates), using 10 males treated with tepa at 10 mg/cm² for 6 hours and mated with 10 untreated females, complete sterility was still not obtained. Larval sterility did increase to 99.20%, and the F_1 adult sterility to 99.99%.

15 mg/cm².--In view of the results of these tests, another was conducted using 10 males treated with tepa at 15 mg/cm² for 6 hours. These males were also mated with 10 untreated females. The larval sterility was 99.99%. This test was terminated 6 days after day 14 because 100%

sterility had not been achieved, and treated male mortality was 10% in the 24-hour post-treatment holding period. In the lower dosage experiments, no treated adult males died during the post-treatment period.

Inability to achieve total male sterility.--Several possibilities present themselves as to why the adult male flea cannot be made totally sterile. Wheeler (1962) states that the alkylating agents (of which tepa is one) can affect directly or indirectly the alkylation of nucleoproteins. Since the alkylation would interfere with mitosis, the rate and replication of deoxyribonucleic acid (DNA) could be altered. Also, DNA is most likely the primary site of alkylation and therefore the most sensitive. Kilgore (1965), working with house flies, reported that the alkylating agents "have a very pronounced effect on the metabolism of the nucleic acids." This is probably also true in X. cheopis, reducing the synthesis of DNA so that the egg cannot form lactic acid dehydrogenase.

In a personal communication (1966), Rothschild confirmed this author's observation that in male flea testes, spermatogenesis is essentially complete when the male emerges from the pupa. At this stage the testis has its full complement of sperm. There is no germinal region in the adult male. Since spermatogenesis is either completed or almost completed upon emergence, the metabolism of the sperm in the testis is probably quite low. In other words, transport of nutrients and gas exchange are not actively taking place, as they would be during spermatogenesis. If this is the case, the chemosterilant would probably have to diffuse through the epithelial layer of the testis, and penetrate the sperm heads.

Keiser et al. (1965) state that in chemosterilized fruit fly males both the spermatogonia and the spermatocytes are destroyed, but that the spermatids which are beyond the last division continue to develop and to mature.

It would seem that certain individual sperm have probably completed spermatogenesis, thus being unaffected by the alkylation of the DNA, while the remaining 99.99% of the sperm have been affected. This would cause the sperm inactivation described by LaChance (1967) and, therefore, the absence of pronuclear fusion, producing sterile eggs.

Sperm transferred by tepa-treated males during copulation is probably inactivated sperm or sperm which contains dominant lethal mutations. Aspermy has certainly not taken place in such a case, since sperm were found motile in the spermatheca of the females at the termination of 14-day experiments.

Oviposition in these experiments was not affected when treated males were mated with untreated females. This condition was also reported by Mitlin et al. (1957) in the house fly. Bertram (1963) observed that even up to 32 days after treatment with thio-tepa the males of A. aegypti had active sperm, and that these sperm were abundant in the untreated female spermathecae. Fahmy and Fahmy (1964) observed that untreated Drosophila females, mated to males treated with varying dosages of the chemosterilant tretamine (TEM), had a very high number of unhatched eggs. Rai (1964) reported that sperm of male A. aegypti treated with apholate produced dominant lethal mutations, since egg hatch was extremely low. The chemosterilant might cause a mutation which acts as a gametic lethal, or as an early zygotic lethal (Fahmy and Fahmy, 1964).

Apparently there are two types of sperm transferred by tepa-treated X. cheopis males. With the first (inactivated sperm), embryonic development does not take place, and the egg color remains essentially the same. With the second type of sperm, however, embryonic development does take place, but the embryos cease growth at various stages of development, and die (dominant lethal mutation). In some of the eggs setae, head capsules, and segmentation of larvae can be seen. In others there appear to be healthy larvae; however, they never emerge.

Dominant lethal mutations seem to affect not only the embryo, but the larvae as well. Upon emerging, larvae from the treated fleas in these experiments did not appear to differ from the control larvae. They were active, and readily burrowed into the larval media, as did the controls. Yet, as can be seen from Table 4, the percent sterility of F_1 adults from larvae of treated males is higher than that of the emerging larvae. Therefore, some of these larvae were certainly affected. It was not determined at what stage of development the larvae died, but it would be interesting to know at exactly which stage death occurred. This could be done by rearing the larvae in artificial media according to the method of Pausch (1962).

Treated females X treated males

The percent sterility obtained from the mating of treated fleas with untreated fleas indicated that more efficient results might be obtained when both sexes were treated with tepa. Therefore, 10 females treated with tepa at 5 mg/cm^2 for 6 hours were mated with 10 males treated with tepa 10 mg/cm^2 for 4 hours. The females laid a few

eggs during the first 3 days of the experiment, after which no more were laid. No larvae were produced.

With such results, two factors seem to be involved. First, as can be seen in Fig. 16, the ovarioles were almost destroyed. The remnant of one oöcyte can be seen (A), and these ovarioles are also leathery, opaque, and white in color, as well as being vacuolated. Second, the sperm from the male must have been inactivated by tepa, since the treated male testes, as well as the spermathecae of the females, contained motile sperm.

Cytological effects of tepa

Since no work had been done previously on the cytological effects of radiation or chemosterilization of X. cheopis, it has been essential to incorporate descriptions and terminology of other authors on other insects in order to interpret the results on X. cheopis.

Bayreuther (1954) was the first to report the chromosome number in X. cheopis from meiotic division. The female flea has $2n=18$ chromosomes with the sex chromosome being $X_1X_1X_2X_2$. The male flea has $2n=17$ chromosomes with the sex chromosomes being trivalent X_1X_2Y , as in the Mantids.

The mitotic chromosomes of X. cheopis are J-shaped (atelomitic) or V-shaped (metacentric), in the terminology of White (1951). These shapes can be seen in Fig. 17.

Brains from treated larvae were examined for chromosomal aberrations; however, no changes were observed after 24 hours. Another sample, examined 48 hours after treatment, showed chromosomal aberrations.



Fig. 16.--Ovarioles of female fleas treated with tepa at 5 mg/cm^2 for 6 hours. (A) remnant of oocyte. 130x.



Fig. 17.--Untreated female mitotic metaphase chromosomes ($2n=18$) from the brain of the prepupa. 1667x.

The terminology used by Rai (1964) in describing chromosomal aberrations in A. aegypti is usually applied here to describe the mitotic effects of tepa on the flea. He grouped the induced aberrations into three principal categories: physiological and structural aberrations, and miscellaneous effects which include induction of somatic polyploidy and probable interference with the normal replication mechanism.

In Fig. 18, two types of aberrations can be observed in the 4 chromosomes visible (all others having been squashed out of the field of view). The first, physiological, is chromosome stickiness. Stickiness is most pronounced at the chromosome ends. The second, structural, could not be found in Rai's descriptions; however, Catcheside (1948) has diagrammed chromosome structural changes induced by radiation. According to his description this tepa-induced aberration would appear to be an inter-arm chromatid exchange aberration.

Chromosomal constriction seems to be visible in Fig. 19. This condition might be due either to the ends sticking to each other, or to fusion at two broken ends.

In Fig. 20 there can be readily observed fragments similar to those shown by Purdon (1963), and there appears to be an isodiametric fragment similar to the type noted by Catcheside (1948). A dicentric chromosome is present. There appear to be many recombinations, rather than deletions as photographed by Murray and Bickley (1964) with apholate on C. p. quinquefasciatus, and by Flint (1964) with radiation on H. pusio.

It would seem, then, that tepa induces non-random breaks. As with apholate, tepa may also break the DNA core, and yet not completely break the chromosome matrix envelope.



Fig. 18.--Metaphase chromosomes from tepa-treated larval brain. (A) chromosome stickiness; (B) inter-arm chromatid exchange aberration. 1667x.



Fig. 19.--Metaphase chromosomes from tepa-treated larval brain. (A) chromosomal constriction. 1667x.



Fig. 20.--Metaphase chromosomes from tepa-treated larval brain. (A) chromosomal fragments; (B) isodiometric fragment; (C) dicentric chromosome. 1667x.

Plapp et al. (1962) reported that with the chemosterilant P^{32} , labelled methaphoxide (tris(2-methyl-1-aziridinyl)phosphine oxide), there was no difference in the metabolism of this compound in organophosphate-resistant and -susceptible house flies. This finding is supported by Wheeler (1962) who states that as yet no mechanism of resistance to alkylating agents has been definitely established.

Field application

Biology.--The potential egg production of X. cheopis females can be determined using the results obtained from the predicted egg production curve in Fig. 8. After flea population counts have been made in the field, the size of future populations can be predicted. Thus, one would know when measures for control of the flea would be most appropriate for maximum effectiveness.

Using the rearing method described herein, one could determine the effectiveness of a chemosterilant by the percentage of F_1 emergence from field-collected specimens.

Chemosterilization.--For practical field application of a sterility program, one essential factor is that the sterilized insects be able to mate with non-sterile individuals. Even though chemosterilant mortality would reduce a population, it would be better to have sterilized individuals live and mate with the non-sterile ones.

Now that sterility with tepa has been achieved in X. cheopis, it should be determined if this flea can become sterilized by feeding on tepa-fed rats or mice. If so, bait stations with the chemosterilant could be set up for the eradication of rodents and their ectoparasites.

SUMMARY

The female X. cheopis can be completely sterilized with tepa at a dosage of 10 mg/cm^2 for a contact treatment time of 4 hours. The ovarioles are virtually destroyed with only remnants left. Treatment of females with a dosage of 5 mg/cm^2 for 6 hours gave 99.28% sterility based on the emergence of F_1 progeny.

Better than 99.99% sterility could not be achieved in males at dosages of 5, 10, or 15 mg/cm^2 for 4 and for 6 hours.

When females treated for 6 hours with tepa at 5 mg/cm^2 were mated with males treated with tepa at 10 mg/cm^2 for 4 hours, 100% sterility was achieved.

Larvae ready to pupate were treated with tepa at 10 mg/cm^2 for 4 hours. Forty-eight hours after treatment, tepa-induced chromosomal aberrations were found in the larval brain. These aberrations appeared to be: (1) chromosome stickiness; (2) inter-arm chromatid exchange; (3) chromosomal constriction; (4) chromosomal fragments; (5) isodimetric fragments; and (6) dicentric chromosome. These aberrations were compared with untreated larval brains in mitotic division.

It was found that the females of the Orlando strain of X. cheopis have 3, 4, or 6 ovarioles per ovary.

In 12 experiments lasting 14 days, the average number of eggs laid daily per female was $4.25 \pm .21$; the highest was 12.6.

Blood meals are necessary for egg-laying.

Eggs of mated females are sticky and adhere to the substrate, as do bits of food. Mating appears to stimulate a gland in the female to produce this sticky substance which is lacking in the eggs of virgin females.

Four out of 149 eggs laid by virgin females underwent partial embryonic development, but died before hatch (rudimentary parthenogenesis).

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APPENDIX

Table 5.--Five untreated females mated with five untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	5	5	3	3
2	23	19	11	1
3	23	21	15	10
4	25	21	12	1
5	30	21	15	5
6	26	24	14	0
7	56	47	26	15
8	41	37	21	9
9	27	27	24	14
10	34	33	26	21
11	39	38	16	10
12	42	42	38	38
13	31	31	21	20
14	30	26	20	18
Totals:	432	392	262	165

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 6.--Five males treated with tepa (5mg/cm² for 6 hrs.) and mated with five untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	17	15	0	0
2	18	17	0	0
3	16	15	0	0
4	18	16	0	0
5	31	30	0	0
6	19	15	0	0
7	34	34	1	1
8	38	36	0	0
9	28	28	0	0
10	18	18	0	0
11	38	37	0	0
12	21	21	0	0
13	34	33	0	0
14	24	24	2	0
Totals:	354	339	3	1

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 7.--Ten untreated females mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	9	9	9 ^c	7
2	22	21	18	18
3	30	30	25	25
4	22	21	14	14
5	35	35	28 ^d	27
6	19	19	14	11
7	23	23	20	18
8	35	34	29	29
9	39	37	37	35
10	27	25	16	16
11	32	32	31	28
12	38	31	26	24
13	35	32	23	20
14	47	38	28	26
Totals:	413	387	318	298

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

^cTwo larvae died.

^dOne larva died.

Table 8.--Ten untreated females mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	4	3	3 ^c	--
2	29	29	24	20
3	80	70	59	51
4	48	39	28	17
5	49	49	32	28
6	51	51	41	35
7	41	41	38	34
8	22	20	15	14
9	32	32	28	27
10	50	48	44	40
11	21	20	14	12
12	29	27	16	16
13	52	46	41	38
14	50	50	46	45
Totals:	558	525	429	377

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling..

^cAll larvae died.

Table 10.--Ten untreated females mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	P ₁ Adults
1	30	28	19	0
2	40	40	9	6
3	56	56	49	44
4	52	52	46	40
5	62	60	43	42
6	65	62	51	48
7	97	96	77	75
8	50	49	42	34
9	91	91	67	54
10	80	79	68	56
11	91	89	79	76
12	92	91	72	72
13	80	76	68	55
14	80	77	58	55
Totals:	966	946	748	657

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 9.--Ten untreated females mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	8	8	7	7
2	34	34	31	30
3	42	42	38	34
4	54	52	42	38
5	55	54	44 ^c	40
6	40	36	26	23
7	62	62	57	54
8	53	53	43	40
9	36	36	30 ^c	26
10	53	52	35	33
11	42	42	34	32
12	51	51	38	37
13	28	28	27	27
14	54	51	47	44
Totals:	612	601	499	465

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

^cOne larva died.

Table 11.--Ten females treated with tepa (5mg/cm² for 4 hrs.) and mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	8	8	6	1
2	10	10	9	0
3	14	14	8	2
4	3	3	1	1
5	6	6	4	0
6	38	37	24	6
7	22	22	18	2
8	19	19	15	13
9	26	25	22	5
10	39	37	28	21
11	23	22	19	15
12	24	23	16	12
13	45	45	38	9
14	24	24	20	18
Totals:	301	295	228	105

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 12.--Ten females treated with tepa (5mg/cm² for 6 hrs.) and mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	4	4	0	0
2	22	22	14	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	5	5	3	0
11	7	7	7	2
12	9	6	2	1
13	13	13	10	7
14	24	24	13	11
Totals:	84	81	49	21

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 13.--Ten females treated with tepa (10mg/cm² for 4 hrs.) and mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	5	5	0	0
2	12	11	1	0
3	5	5	1	0
4	3	3	1	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
Totals:	25	24	3	0

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were broken, or were broken in handling.

Table 14.--Ten females treated with tepa (10mg/cm² for 4 hrs.) and mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived	Larvae	F ₁ Adults
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
Totals:	0	0	0	0

^aControl fleas began egg production on the 3rd day after being placed on the host.

Table 15.--Ten females treated with tepa ($10\text{mg}/\text{cm}^2$ for 4 hrs.) and mated with ten untreated males.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived	Larvae	F ₁ Adults
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
Totals:	0	0	0	0

^aControl fleas began egg production on the 3rd day after being placed on the host.

Table 16.--Ten males treated with tepa ($5\text{mg}/\text{cm}^2$ for 6 hrs.) and mated with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	7	6	0	0
2	24	24	2	0
3	36	36	0	0
4	56	56	1	1
5	57	56	1	0
6	30	30	0	0
7	37	37	0	0
8	53	53	0	0
9	64	64	1	0
10	40	39	0	0
11	56	56	0	0
12	45	45	0	0
13	53	53	0	0
14	50	50	0	0
Totals:	608	605	5	1

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 17.--Ten males treated with tepa (5mg/cm² for 6 hrs.) and mated with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	10	10	0	0
2	31	31	0	0
3	30	30	1	0
4	30	28	0	0
5	64	62	0	0
6	30	29	0	0
7	54	54	0	0
8	40	39	0	0
9	55	55	0	0
10	36	35	0	0
11	40	40	0	0
12	40	40	0	0
13	55	55	0	0
14	54	54	0	0
Totals:	569	562	1	0

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 18.--Ten males treated with tepa ($5\text{mg}/\text{cm}^2$ for 6 hrs.) and mated with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	19	19	1	0
2	27	27	0	0
3	37	36	1	0
4	41	39	0	0
5	40	40	0	0
6	35	35	1	1
7	25	25	0	0
8	47	47	0	0
9	72	72	0	0
10	68	68	0	0
11	73	73	1	0
12	70	70	0	0
13	46	46	0	0
14	54	54	0	0
Totals:	654	651	4	1

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 19.--Ten males treated with tepa (10mg/cm² for 4 hrs.) and crossed with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	6	6	0	0
2	38	38	1	0
3	39	34	2	0
4	26	23	1	0
5	43	43	3	0
6	60	55	7	0
7	52	46	6	1
8	35	33	5	0
9	33	32	2	0
10	37	33	3	0
11	39	39	7	4
12	57	57	7	3
13	40	40	9	0
14	47	44	8	3
Totals:	552	523	61	11

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 20.--Ten males treated with tepa (10mg/cm² for 6 hrs.) and mated with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	18	18	0	0
2	41	40	0	0
3	22	21	0	0
4	25	25	0	0
5	40	40	3	2
6	49	49	0	0
7	41	41	0	0
8	43	43	0	0
9	21	21	0	0
10	38	38	0	0
11	33	33	0	0
12	27	27	0	0
13	32	32	0	0
14	26	26	0	0
Totals:	456	454	3	2

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling..

Table 21.--Ten males treated with tepa (10mg/cm² for 6 hrs.) and mated with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	5	5	0	0
2	30	29	0	0
3	65	65	0	0
4	48	48	0	0
5	47	47	0	0
6	73	73	0	0
7	49	49	0	0
8	48	48	0	0
9	44	44	1	0
10	33	33	0	0
11	18	18	0	0
12	30	30	1	1
13	48	48	0	0
14	55	55	0	0
Totals:	593	592	2	1

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 22.--Ten males treated with tepa (10mg/cm² for 6 hrs.) and mated with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	16	16	1	0
2	30	29	0	0
3	32	32	1	0
4	48	47	0	0
5	54	54	1	0
6	47	47	0	0
7	69	68	1	1
8	55	53	0	0
9	49	49	0	0
10	22	21	0	0
11	55	55	0	0
12	63	63	0	0
13	78	78	0	0
14	96	96	0	0
Totals:	714	708	4	1

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

Table 23.--Ten males treated with tepa (15mg/cm² for 6 hrs.) and mated with ten untreated females.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults ^c
1	5	5	0	--
2	28	28	0	--
3	33	31	0	--
4	36	34	0	--
5	45	45	0	--
6	50	50	1	--
7	38	38	0	--
8	31	31	1	--
9	35	35	0	--
10	20	20	0	--
11	29	29	0	--
12	32	32	0	--
13	33	33	0	--
14	37	37	0	--
Totals:	452	448	2	--

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

^cExperiment was terminated 6 days after Day 14 because 100% sterility had not been achieved, and treated male mortality was 10% in the 24-hour post-treatment holding period.

Table 24.--Ten females treated with tepa (5mg/cm² for 6 hrs.) and mated with ten males treated with tepa at 10mg/cm² for 4 hrs.

Day ^a	NUMBER OF			
	Eggs		Emerging	
	Produced	Survived ^b	Larvae	F ₁ Adults
1	1	1	0	0
2	5	4	0	0
3	4	3	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
Totals:	10	8	0	0

^aEggs were first observed on the 3rd day after placing fleas on host.

^bSome eggs were found broken, or were broken in handling.

BIOGRAPHICAL SKETCH

Robert Loomis Linkfield was born on October 28, 1930, at Jamaica, Long Island, New York. In 1949, after graduation from Bayside High School, he began a two-year course of study at Long Island Agricultural and Technical Institute, from which he received the degree of Associate of Applied Science in 1951. From then until 1953 he served with the United States Army as a Sound Ranging Specialist (GR8), with eleven months in Korea during the police action there.

In June of 1956 he received the degree of Bachelor of Science from the University of Georgia, and was awarded the degree of Master of Science (Entomology) in June of 1957, after which he was employed by the United States government.

After a year with the Agency for International Development, for which he worked as junior overseas officer trainee, he was transferred in 1959 to the United States Department of Agriculture, Agricultural Research Service, Regional Insect Control Project, with which he was associate entomologist for a year, and entomologist-in-charge for three years in Iran and Libya.

With the help of a graduate assistantship at the J. Hillis Miller Medical Center where he is in charge of pest control for the medical science complex, he enrolled in the Graduate School at the University of Florida. From September 1963 to the present he has worked toward the degree of Doctor of Philosophy.

Married to the former Ethel Skelton, he is the father of a daughter. He is a member of the Society of the Sigma Xi; Alpha Zeta; Phi Sigma; Newell Entomological Society; Florida Entomological Society; and the Washington Entomological Society.

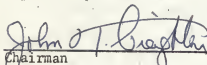
This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

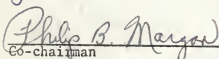
December, 1966


Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:


Chairman


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